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(54) PHYSIOLOGICALLY ACTIVE SUBSTANCE EEM-S ORIGINATING IN MUSHROOMS, PROCESS FOR PRODUCING THE SAME AND DRUGS

(57) EEM-S obtained by extracting with hot water or a lower alcohol at least one mushroom selected from among *Lentinus edodes, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pholiota nameko, Grifola fondosa, Volvariella speciosa ver. speciosa, Lyophllum decastes, blanc du pays, Tricholoma matsutake, Ganoderma lucidum* and *Phellinus yucatensis,* treating the obtained extract by the molecular sieve method and thus eliminating low-molecular-weight and high-molecular-weight fractions: and preparations thereof. This EEM-S exerts physiological effects such as anticancer, immunopotentiating, antioxidative, hypotensive and hypoglycemic effects.

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Description

TECHNICAL FIELD

[0001] The present invention relates to a substance having biological activity, such as anticancer activity, antiallergic activity, immunopotentiating activity. and antioxidation activity, obtained from at least one edible mushroom selected from *Lentinus edodes, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Grifola fondosa, Volvariella speciosa ver. speciosa, Pholiota nameko, Lyophllum decastes, Blanc du pays, Tricholoma matsutake,* and *Bracket fungi* such as *Ganoderma lucidum* and *Phellinus yucatensis* (*Phellinus linteus*), a process for producing the substance, and a preparation containing the biologically active substance.

BACKGROUND ART

[0002] The present inventors have conducted extensive studies on biological activity of mushrooms for many years. It is known in the art that some species of mushrooms exhibit biological activity such as anticancer effects, immunopotentiating effects, antioxidative effects, hypotensive and hypoglycemic effects. However, mushrooms haying no or only a small degree of activity are also advertised as being very efficacious and put on the market.

[0003] The present inventors have found that the anticancer effects of mushrooms originate from polysaccarides contained therein from the research results based on a mass of research data, and have conducted studies on a glucan which is the polysaccaride contained in mushrooms. However, the present inventors have found that the glucan, which is a pure simple polysaccaride, exhibits activity by injection, but does not exhibit effects by oral administration.

[0004] Since the administration by injection can be carried out only in the hospital or the like and oral administration causes a person less pain, provision of active components from mushrooms effective through oral administration has been strongly demanded.

[0005] Accordingly, an object of the present invention is to provide a method for efficiently extracting a biologically active substance exhibiting biological activity such as anticancer activity and immunopotentiating activity through oral administration from mushrooms containing a high concentration of such a substance, and a preparation containing the biologically active substance suitably prepared for use.

DISCLOSURE OF THE INVENTION

[0006] As a result of extensive studies to achieve the above object, the present inventors have found that fruit bodies and/or mycelia of ediblemushrooms contain a large amount of active substances effective through oral administration. The active substances effective through oral administration are contained in an extract of the mushrooms with water, a hydrophilic solvent, or a mixed solvent of these. The present inventors have found that a fraction having a specific molecular weight range obtained by removing low-molecular-weight and high-molecular-weight fractions from the extract using a molecular sieve method exhibits excellent biological activity. This finding has led to the completion of the present invention.

[0007] Specifically, the present invention provides a biologically active substance EEM-S obtained by extracting at least one mushroom selected from *Lentinus edodes, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pholiota nameko, Grifola fondosa, Volvariella speciosa ver. speciosa, Lyophllum decastes, Blanc du pays, Tricholoma matsutake, Ganoderma lucidum,* and *Phellinus yucatensis* (*Phellinus linteus*), with water, a hydrophilic solvent, or a mixed solvent of these, and collecting a fraction having a molecular weight of 6,000-60,000 from the extract using a molecular sieve method.

[0008] The present invention also provides a process for producing the biologically active substance EEM-S.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 is a view showing an ultraviolet and visible absorption curve of a biologically active substance EEM-SB obtained in Example 1, in which the solvent is water.

[0010] FIG. 2 is a view showing an ultraviolet and visible absorption curve of a biologically active substance EEM-SS obtained in Example 2, in which the solvent is water.

[0011] FIG. 3 is a view showing an ultraviolet and visible absorption curve of a biologically active substance EEM-SM obtained in Example 3, in which the solvent is water.

[0012] FIG. 4 is a view showing an ultraviolet and visible absorption curve of a biologically active substance EEM-SN obtained in Example 4, in which the solvent is water.

[0013] FIG. 5 is a view showing an ultraviolet and visible absorption curve of a biologically active substance EEM-SE obtained in Example 5, in which the solvent is water.

[0014] FIG. 6 is a view showing an infrared absorption curve of the biologically active substance EEM-SB obtained in Example 1 measured using an ATR method.

[0015] FIG. 7 is a view showing an infrared absorption curve of the biologically active substance EEM-SE obtained in Example 5 measured using an ATR method.

[0016] FIG. 8 is a chart showing ^{1}H-NMR (D_2O) of the biologically active substance EEM-SB obtained in Example 1.

[0017] FIG. 9 is a chart showing ^{1}H-NMR (D_2O) of the biologically active substance EEM-SE obtained in Example 5.

[0018] FIG. 10 is a chart showing ^{13}C-NMR (D_2O) of the biologically active substance EEM-SB obtained in Example 1.

[0019] FIG. 11 is a chart showing ^{13}C-NMR (D_2O) of the biologically active substance EEM-SE obtained in Example 5.

BEST MODE FOR CARRYING OUT THE INVENTION

[0020] The biologically active substance EEM-S of the present invention is obtained by extracting, with water and/or a hydrophilic solvent, a finely ground product of fruit bodies and/or mycelia of at least one edible mushroom such as *Lentinus edodes, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pholiota nameko, Grifola fondosa, Volvariella speciosa ver. speciosa, Lyophllum decastes, Blanc du pays, Tricholoma matsutake, Ganoderma lucidum,* or *Phellinus yucatensis* (*Phellinus linteus*) (hereinafter referred to as "mushroom"). As the hydrophilic solvent used for extraction, a lower alcohol having 1-4 carbon atoms is preferably used.

[0021] A fraction containing the biologically active substance EEM-S is extracted from the mushroom by adding an appropriate amount of water or a hydrophilic solvent such as a lower alcohol to the raw material mushroom, and extracting the fraction under reflux at a temperature of 80-98°C, and preferably 90-98°C in the case of using water, for about 1-4 hours, and preferably 2-4 hours, for example. The amount of solvent used for extraction is about three to ten times the weight of the mushroom.

[0022] After optionally separating solid impurities from the resulting extract by filtration or the like, the solvent is evaporated under reduced pressure to obtain a solid extract.

[0023] The solid extract thus obtained exhibits activity through oral administration and may be used as is. However, the activity can be increased by using a fraction having a molecular weight of 6,000-60,000 obtained by removing an unnecessary portion from the extract using a molecular sieve and concentrating the resulting product under reduced pressure as the biologically active substance EEM-S.

[0024] As the molecular sieve used for the above purpose, a molecular sieve membrane (flat membrane), hollow filter membrane, permeable membrane, molecular sieve chromatography, and the like can be given. The biologically active substance EEM-S may be separated by appropriately applying a method using these molecular sieves.

[0025] A method of separating the biologically active substance EEM-S is described below taking a case of using a module-type hollow filter membrane as an example. The extract of the mushroom with water and/or a hydrophilic solvent (fraction containing the biologically active substance EEM-S) is treated using a low-molecular-weight fraction membrane module such as AIP-3013, AIP-2013, or AIP-1010 (manufactured by Asahi Kasei Corporation) to remove low-molecular-weight substances (substances having a molecular weight of 6,000 or less). The concentrate from which the low-molecular-weight substances are removed is treated using a high-molecular-weight fraction membrane module such as AHP-3013, AHP-2013, or AHP-1010 (manufactured by Asahi Kasei Corporation) to remove high-molecular-weight substances (substances having a molecular weight of 60,000 or more). The resulting solution is concentrated using a low-molecular-weight fraction membrane module. A biologically active substance EEM-S having a molecular weight of 6,000-60,000 is obtained in this manner.

[0026] In the case of using the molecular sieve method, the molecular weight may differ from the molecular weight determined using other methods, since the substances are sifted differently depending upon the shape of the molecules. In the present invention, the molecular weight refers to a molecular weight determined using the molecular sieve method. More precisely, the molecular weight on the low-molecular-weight side is a value measured using AIP-3013 or a low-molecular-weight fraction membrane module equal to AIP-3013. Similarly, the molecular weight on the high-molecular-weight side is a value measured using AHP-3013 or a high-molecular-weight fraction membrane module equal to AHP-3013.

[0027] The biologically active substances of the mushrooms thus obtained are collectively referred to as EEM-S. However, in the case of using the mycelia of the mushrooms as the raw material, the yield of the biologically active substance varies depending upon culture conditions. Therefore, it is necessary to select an optimum culture medium, culture temperature, and the like. Moreover, physicochemical properties may differ depending upon the species of mushrooms.

[0028] For example, the biologically active substance EEM-S produced using *Hypsizygus marmoreus* as the raw material (hereinafter may be called "EEM-SB") exhibits physicochemical properties shown in Example 1. The biologically active substance EEM-S produced using *Flammulina velutipes* as the raw material (hereinafter may be called "EEM-SE") exhibits physicochemical properties shown in Example 5. The biologically active substances EEM-S produced using *Lentinus edodes, Grifola fondosa,* and *Pholiota nameko,* respectively, as the raw material (hereinafter

may be called "EEM-SS", "EEM-SM", and "EEM-SN", respectively) have ultraviolet and visible absorption shown in FIGS. 1 to 5.

[0029] Although there are small differences in the physicochemical properties, these biologically active substances exhibit high biological activity in comparison with a simple hot water extract of each mushroom, as shown in Example 6. Therefore, these biologically active substances are considered to have common properties.

[0030] The above-described biologically active substance EEM-S exhibits biological activity through oral administration. The dose of EEM-S through oral administration to obtain the predetermined effects differs depending upon the age and the weight of a person, purpose, and the like. The dose of EEM-S is generally 200-5000 mg, and preferably 1000-3000 mg per day for an adult. It is suitable that the above amount be separately administered several times per day.

[0031] The biologically active substance EEM-S of the present invention may be prepared as a powdered preparation, granule preparation, capsule preparation, or liquid preparation using a conventional method. However, since the biologically active substance EEM-S may be discolored or deteriorate when allowed to stand in air due to absorption of moisture, it is preferable to use the biologically active substance EEM-S as a tablet provided with a film coat. As the film coat material, a soybean peptide, a shell resin material, and the like are preferable. Absorption of moisture may not be securely prevented using other film coat materials, whereby the biologically active substance may be discolored or deteriorate.

EXAMPLES

[0032] The present invention is described below in more detail by examples. The following examples take anticancer effects as an example of the biological activity. However, the biological activity of the substance of the present invention is not limited to the anticancer effects. The substance of the present invention has the above-described biological activity.

Example 1

[0033] 1000 g of *Hypsizygus marmoreus* was finely ground and extracted with hot water. A precipitate was removed from the resulting extract using a PS-88 membrane (manufactured by Ohtsuka Jitsugyo Co., Ltd.). A fraction containing high-molecular-weight substances (molecular weight of 6, 000 or more) was concentrated from the hot water extract using a module-type hollow filter membrane for low-molecular-weight fractions ("AIP-3013" manufactured by Asahi Kasei Corporation). High-molecular-weight substances (molecular weight of 50,000 or more) were removed from the concentrate using a module-type hollow filter membrane for high-molecular-weight fractions ("AHP-3013" manufactured by Asahi Kasei Corporation). The resulting solution was concentrated using a low-molecular-weight fraction membrane module AIP-3013. A biologically active substance EEM-SB having a molecular weight of 6, 000-50,000 was obtained by the above continuous treatment.

<Physicochemical properties of EEM-SB>

[0034]

(1) Ultraviolet absorption (UV absorption at 370-190 nm in water was measured): maximum value: 258.0 nm (absorption curve is shown in FIG. 1)
(2) Infrared absorption (measured using ATR method): maximum value (cm^{-1}) : 218, 1560, 1396, 1032 (absorption curve is shown in FIG. 6)
(3) ^{1}H-NMR (measured in D_2O): FIG. 8 shows the chart.
(4) ^{1}C-NMR (measured in D_2O): FIG. 10 shows the chart.
(5) Protein content : 24.3%
(6) Carbohydrate content: 27.0%
(7) Ratio of essential carbohydrates: glucose:galactose:mannose = 18:2:1
(8) Amino acid composition: aspartic acid; 9.0%, glutamic acid; 16.1%, glycine; 9.1%, alanine; 12.5%, valine; 5.7%, arginine; 5.6%, ornithine; 7.8%
(9) Elementary analysis value: C: 36.2%, H: 6.0%, N: 5.8%

[0035] The EEM-SB thus obtained can be powdered by freeze-drying. A tablet which can be orally administered is formed by tableting the powder.

Example 2

[0036] 500 g of *Lentinus edodes* was ground and extracted with hot water. A precipitate was removed from the resulting extract using a PB-88 membrane (manufactured by Ohtsuka Jitsugyo Co., Ltd.). A fraction containing high-molecular-weight substances was concentrated from the hot water extract using a module-type hollow filter membrane for low-molecular-weight fractions ("AIP-3013" manufactured by Asahi Kasei Corporation) . High-molecular-weight substances were removed from the concentrate using a module-type hollow filter membrane for high-molecular-weight fractions ("AHP-3013" manufactured by Asahi Kasei Corporation). The resulting solution was concentrated using a low-molecular-weight fraction membrane module AIP-3013. The ultraviolet absorption curve of the resulting biologically active substance EEM-SS in water is shown in FIG. 2.

[0037] The EEM-SS thus obtained can be powdered by freeze-drying. A tablet can be formed by tableting the powder.

Example 3

[0038] 1000 g of *Grifola fondosa* was extracted with hot water. The extract was filtered by means of suction using a molecular sieve membrane ("PM-2" manufactured by Ohtsuka Jitsugyo Co., Ltd.) to remove fungus bodies. A low-molecular-weight fraction (molecular weight of 6,000 or less) was removed by dialysis with running water using a permeable membrane. A high-molecular-weight fraction was removed from the resulting solution using a hollow molecular sieve membrane (AHP-3013) to obtain a biologically active substance EEM-SM.

[0039] The ultraviolet absorption curve of the resulting biologically active substance EEM-SM in water is shown in FIG. 3.

Example 4

[0040] 1000 g of *Pholiota nameko* was extracted with hot water. The extract was filtered by means of suction using a molecular sieve membrane ("PM-2" manufactured by Ohtsuka Jitsugyo Co., Ltd.) to remove fungus bodies. A low-molecular-weight fraction (molecular weight of 6,000 or less) was removed by dialysis with running water using a permeable membrane. A high-molecular-weight fraction was removed from the resulting solution using a hollow molecular sieve membrane (AHP-3013) to obtain a biologically active substance EEM-SN.

[0041] The ultraviolet absorption curve of the resulting biologically active substance EEM-SN in water is shown in FIG. 4.

Example 5

[0042] 1000 g of *Flammulina velutipes* was extracted with hot water under reflux to obtain 107 g of an extract. The extract was dissolved in water and dialyzed in a dialysis tube to obtain a fraction containing high-molecular-weight substances (molecular weight of 6,000 or more) in the permeable membrane. The fraction containing high-molecular-weight substances was treated using a molecular sieve membrane PM-10 to remove high-molecular-weight substances (molecular weight of 50,000 or more), and then freeze-dried to obtain a substance containing a high concentration of EEM-SE with a molecular weight of 6,000-50,000. This substance was prepared as a powdered preparation.

<Physicochemical properties of EEM-SE>

[0043] Physicochemical properties of biologically active substance EEM-SE:

(1) Ultraviolet absorption (UV absorption at 370-190 nm in water was measured): maximum value: 258.4 nm (absorption curve is shown in FIG. 5)
(2) Infrared absorption (measured using an ATR method) : maximum value (cm^{-1}) : 3279, 2927, 1578, 1400, 1043 (absorption curve is shown in FIG. 7)
(3) ^{1}H-NMR (measured in D_2O): FIG. 9 shows the chart.
(4) ^{1}C-NMR (measured in D_2O): FIG. 11 shows the chart.
(5) Protein content : 12.7%
(6) Carbohydrate content: 9.8%
(7) Ratio of essential carbohydrates: glucose:galactose:mannose = 16:3:1
(8) Amino acid composition: aspartic acids; 9.1%, glutamic acid; 17.7%, glycine; 14.8%, alanine; 21.9%, valine; 11.8%, arginine; 6.8%, ornithine; 12.6%
(9) Elementary analysis value: C: 36.5%, H: 6.6%, N: 3.5%

Example 6

[0044] A powdered preparation was prepared using the biologically active substance EEM-S obtained in Example 1, 2, 3, or 5, and subjected to the following anticancer test. Anticancer activity was determined by comparing the survival rate between the biologically active substances of the examples and a control group. The results are shown in Table 1.

(Anticancer test)

[0045] Female BDF1 mice were subcutaneously transplanted with viable Lewis lung carcinoma cells. The biologically active substance EEM-S obtained in each example was suspended in purified water and orally administered to the mice at a dose of 500 mg/kg per day for 20 days from the next day. The survival rate was calculated from an average survival period of the mice in a control group in which only purified water was administered, in groups in which a hot water extract of the mushroom was administered, and in groups in which the biologically active substance obtained in Examples 1, 2, 3, or 5 was administered. The results are shown in Table 1.

(Results)

[0046]

TABLE 1

Sample	Average survival period (day)	survival rate (%)
Control group	26.2	
Hypsizygus marmoreus hot water extract	30.2	15.3
Lentinus edodes hot water extract	30.0	14.5
Grifola fondosa hot water extract	29.8	13.9
Flammulina velutipes hot water extract	30.2	15.1
Biologically active substance EEM-SB of Example 1	38.2	45.8
Biologically active substance EEM-SS of Example 2	36.0	37.4
Biologically active substance EEM-SM of Example 3	35.2	34.4
Biologically active substance EEM-SE of Example 5	36.8	40.6

INDUSTRIAL APPLICABILITY

[0047] According to the present invention, substances having high biological activity are easily and efficiently obtained from edible mushrooms, and products useful as drugs or health foods are obtained.

Claims

1. A biologically active substance EEM-S obtained by extracting at least one mushroom selected from *Lentinus edodes, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pholiota nameko, Grifola fondosa, Volvariella speciosa ver. speciosa, Lyophllum decastes, Blanc du pays, Tricholoma matsutake, Ganoderma lucidum,* and *Phellinus yucatensis* (*Phellinus linteus*), with water, a hydrophilic solvent, or a mixed solvent of these, and collecting a fraction having a molecular weight of 6, 000-60, 000 from the extract using a molecular sieve method.

2. The biologically active substance EEM-S according to claim 1, wherein the maximum value of ultraviolet absorption in water is 255-260 nm.

3. The biologically active substance EEM-S according to claim 1 or 2, wherein the hydrophilic solvent is a lower alcohol having 1-4 carbon atoms.

4. A biologically active substance EEM-SB obtained by extracting *Hypsizygus marmoreus* with water, a lower alcohol having 1-4 carbon atoms, or a mixed solvent of these, and treating the extract using a molecular sieve method, the biologically active substance EEM-SB having a molecular weight of 6,000-60,000, a maximum value of ultraviolet absorption in water of 255-260 nm, a maximum infrared absorption wave number of 1560, 1396, and 1032 cm^{-1} (including an error of ±50 cm^{-1}), a protein content of 20-30%, a carbohydrate content of 22-32%, and an

amino acid composition (molar ratio) of aspartic acid; 9.0%, glutamic acid; 16.1%, glycine; 9.1%, alanine; 12.5%, valine; 5.7%, arginine; 5.6%, and ornithine; 7.8% (including an error of ±3%); and containing glucose as an essential constituent carbohydrate.

5. A biologically active substance EEM-SE obtained by extracting *Flammulina velutipes* with water, a lower alcohol having 1-4 carbon atoms, or a mixed solvent of these, and treating the extract using a molecular sieve method, the biologically active substance EEM-SE having a molecular weight of 6,000-60,000, a maximum value of ultraviolet absorption in water of 255-260 nm, a maximum infrared absorption wave number of 3259, 2927, 1578, 1400, and 1043 cm^{-1} (including an error of ±50 cm^{-1}), a protein content of 8-18%, a carbohydrate content of 5-10%, an amino acid composition (molar ratio) of aspartic acids; 9.1%, glutamic acid; 17.7%, glycine; 14.8%, alanine; 21.9%, valine; 11.8%, arginine; 6.8%, ornithine; 12.6% (including an error of ±3%); and containing glucose as an essential constituent carbohydrate.

6. A process for producing a biologically active substance EEM-S, comprising: extracting at least one mushroom selected from *Lentinus edodes, Flammulina velutipes, Hypsizygus marmoreus, Pleurotus ostreatus, Pholiota nameko, Grifola fondosa, Volvariella speciosa ver. speciosa, Lyophllum decastes, Blanc du pays, Tricholoma matsutake, Ganoderma lucidum,* and *Phellinus yucatensis* (*Phellinus linteus*), with water, a hydrophilic solvent, or a mixed solvent of these, and treating the extract using a molecular sieve method to collect a fraction having a molecular weight of 6,000-60,000.

7. The process for producing a biologically active substance EEM-S according to claim 6, wherein the hydrophilic solvent is a lower alcohol having 1-4 carbon atoms.

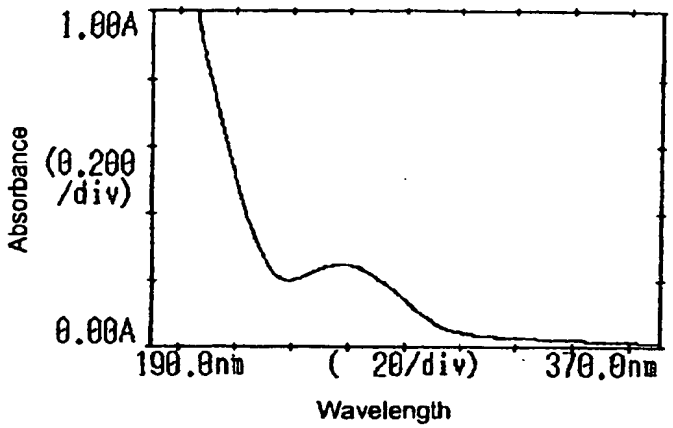
8. The process for producing a biologically active substance EEM-S according to claim 6 or 7, wherein the molecular sieve method uses a molecular sieve membrane (flat membrane), a hollow filter membrane, a permeable membrane, or molecular chromatography.

9. A drug comprising the biologically active substance according to any one of claims 1 to 5 as an active ingredient.

10. The drug according to claim 9, which is an anticancer agent.

11. The drug according to claim 9 or 10, which is in the form of a tablet, powder, granule, capsule, or liquid.

12. The drug according to claim 9, 10, or 11, which is a tablet coated with a soybean peptide and/or shell resin film coat material.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Absorbance
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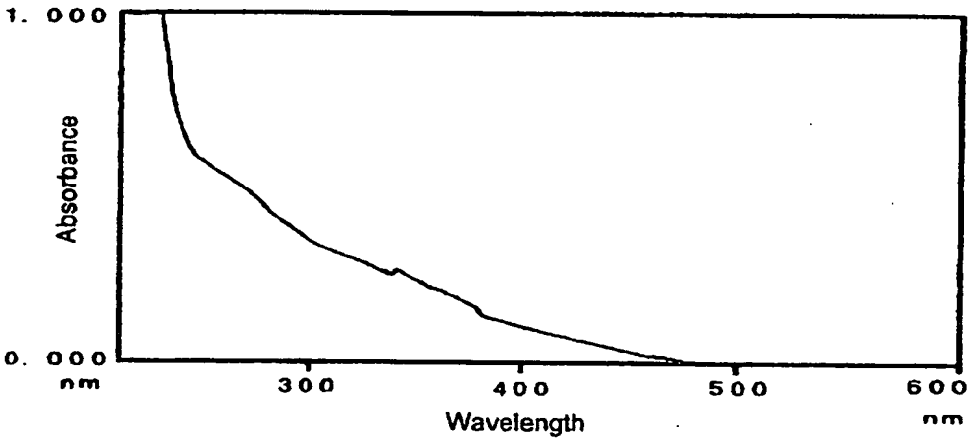
(20/div)
370.0nm
Wavelength

FIG. 1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nm
Wavelength

FIG. 2

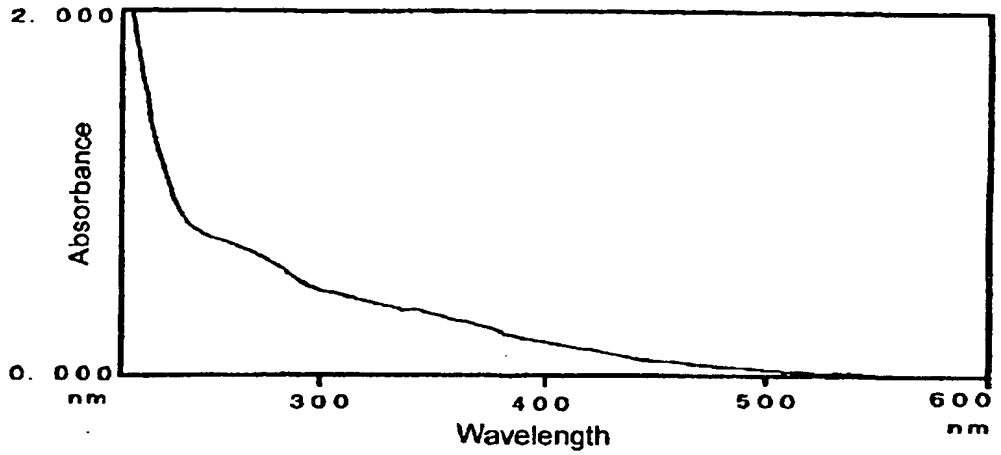

FIG. 3

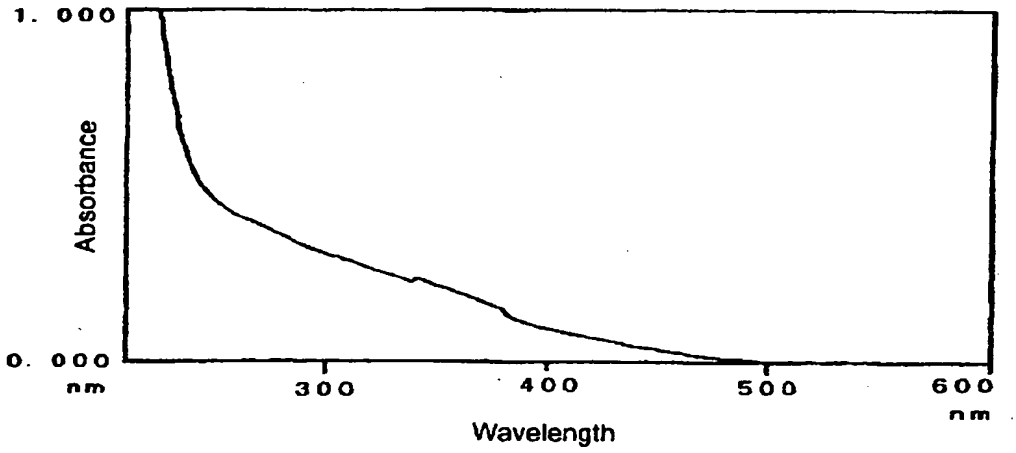

FIG. 4

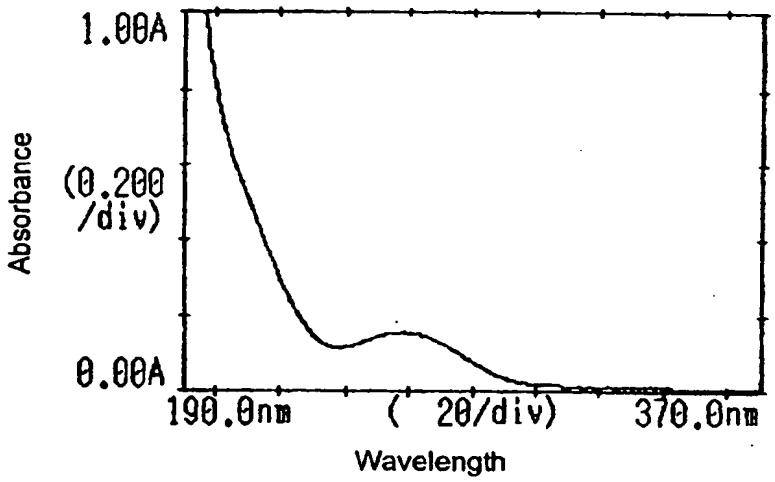

FIG. 5

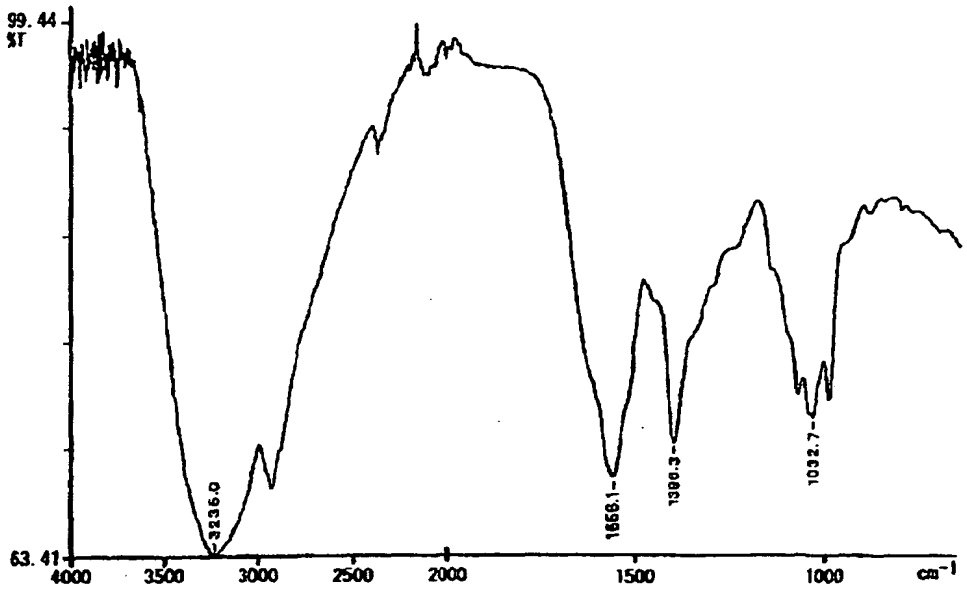

FIG. 6

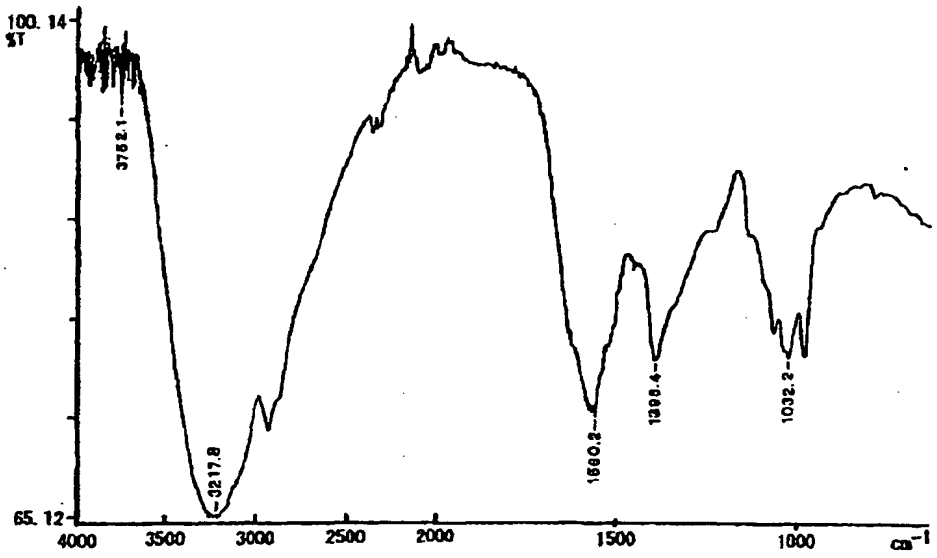

FIG. 7

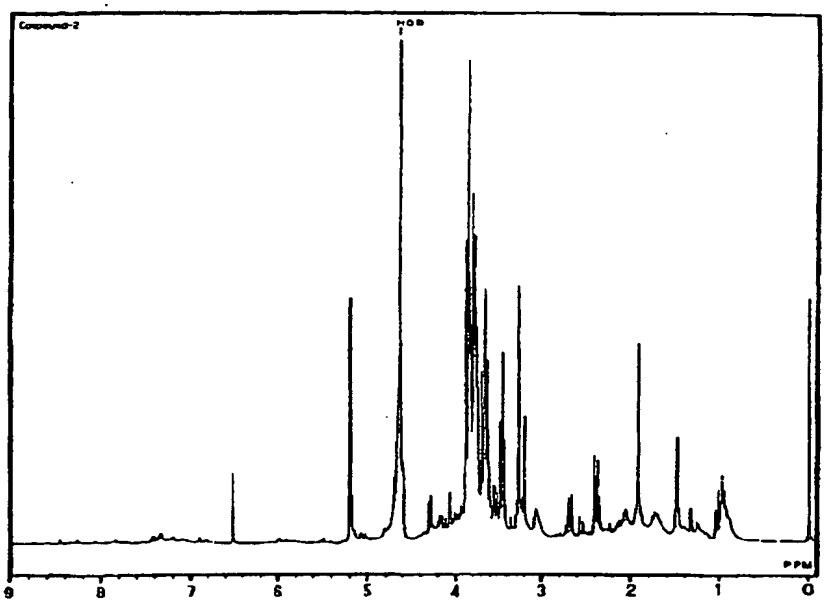

FIG. 8

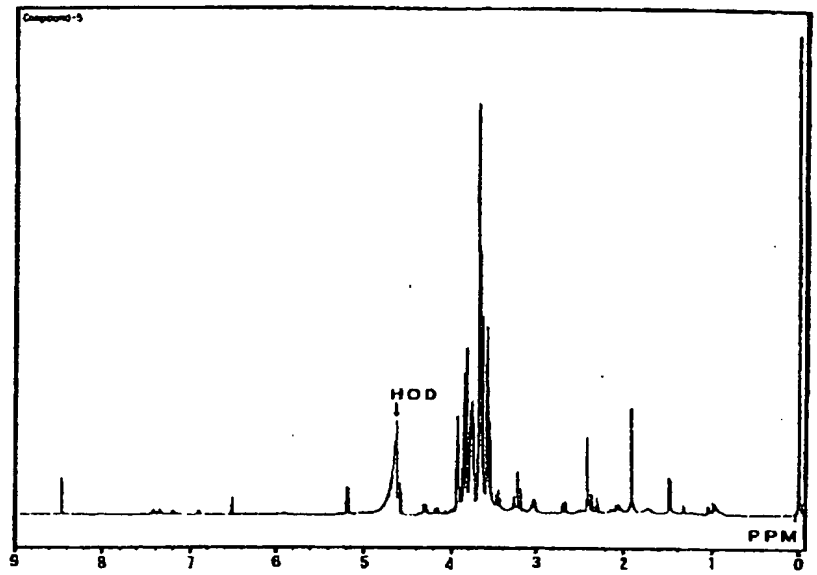

FIG. 9

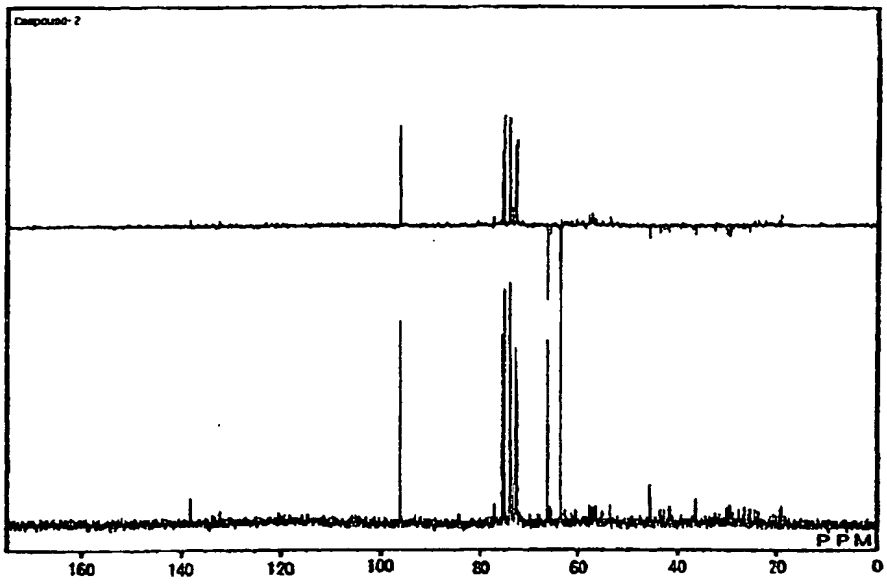

FIG. 10

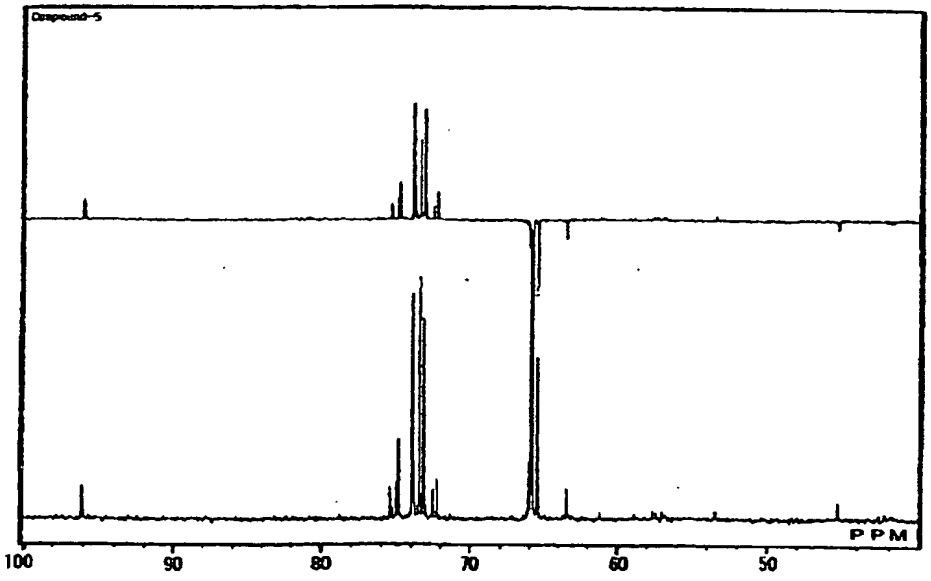

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/00072

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K35/84, 9/20, 9/14, 9/16, 9/48, 9/08, 9/30
A61P35/00, 37/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K35/84, 9/20, 9/14, 9/16, 9/48, 9/08, 9/30
A61P35/00, 37/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA(STN), BIOSIS(STN), MEDLINE(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JP, 2000-159808, A (KOBAYASHI PHARMACEUTICAL Co., Ltd.), 13 June, 2000 (13.06.00) (Family: none)	1,3,6,7-10
X Y	WO, 99/53937, A (EI SHOGEN KK), 28 October, 1999 (28.10.99) & JP, 11-302191, A	1,3,6,7-10 11,12
X Y	JP, 9-315994, A (Sumitomo Forestry Co., Ltd.), 09 December, 1997 (09.12.97) (Family: none)	1,3,6,7-10 11,12
X Y	JP, 63-72629, A (Kyoritsu Yakuhin Kogyo K.K.), 02 April, 1988 (02.04.88) (Family: none)	1,3,6,7-10 11,12
X Y	JP, 8-291078, A (MIO K.K.), 05 November, 1996 (05.11.96) (Family: none)	1,6,8-11 12
X Y	JP, 62-19530, A (Toyo Soda Kogyo K.K.), 28 January, 1987 (28.01.87) (Family: none)	1,6,8-10 11,12
X Y	JP, 56-127317, A (Banyu Pharmaceutical Co., Ltd.), 06 October, 1981 (06.10.81) (Family: none)	1,6,8-10 11,12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 April, 2001 (05.04.01)	17 April, 2001 (17.04.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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